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The Extirpation of the Thyroid Gland and Its Effects upon the Hypophysis in *Bufo americanus* and *Rana pipiens*.

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LITERATURE.

DURING the past decade a great amount of literature has appeared which has quite conclusively proved that the removal of the thyroid gland causes the hypertrophy of the hypophysis. Most of the work has been done on mammals and the observations have been more or less limited to a study of the anterior lobe.

Rogowitsch, in 1886, was the first to show that the removal of the thyroid gland causes the hypophysis to become altered and enlarged. Degener, working at about the same time, found that the increase in weight was directly proportional to the time which had elapsed after the removal of the gland, and that this removal affected all parts of the hypophysis, but more especially the parts anterior. This worker also observed the increase of hyaline and granular masses in the pars intermedia and their passage in large numbers through the pars nervosa into the infundibular extension of the third ventricle. Herring makes the statement that this denotes an increased activity on the part of the pars intermedia.

Rogowitsch, in 1888, removed the thyroid gland in rabbits and dogs and in eight to fourteen days after the operation he found an increase in the size of the hypophysis. Upon a further study of the cells, he found this increase due to an actual increase in the size of the cells, enlargement of the spaces between the nuclei, increase in the number of vacuoles and the amount of colloid in the cell body as well as a marked dilation of the blood vessels.

Hofmeister, in 1892, found that the extirpation of the thyroid gland of young rabbits, the external parathyroids left in situ, was

followed by a retardation of growth and the development of a condition of chronic cachexia. In the internal organs Hofmeister found a condition similar to that described by Rogowitsch in the case of adult rabbits, namely, enlargement of the glandular portion of the hypophysis cerebri and the appearance of large vacuoles in the protoplasm of the enlarged principal cells.

Biedl found an increase of colloid and also that the degree of enlargement varied according to the length of life after the operation. In animals which lived for some time after the removal of the thyroid glands, the hypophysis frequently became two or three times as large as the control. Allen, Adler and Smith have all shown that only the anterior lobe of the hypophysis and the thyroid gland are necessary for the phenomenon of metamorphosis. Allen, in his work on gland implantation, has shown this very conclusively. He finds that a pituitariless tadpole undergoes metamorphosis when the anterior lobe alone of the hypophysis of a mature frog is implanted subcutaneously. Rodger (1918) found that the removal of the thyroid gland increases the size of the anterior lobe of the hypophysis in *Rana pipiens*.

Atwell (1918), in describing the development of the hypophysis of the *Anura*, uses the terms which will be used by the writer of this paper. The same terms are used by Rasmussen (1921) in describing the hypophysis of the woodchuck. The pars intermedia and pars nervosa together are often spoken of as the posterior lobe. The latter term seems a bit unsatisfactory, because the pars nervosa is not of epithelial origin but of neural origin. According to Atwell, the hypophysis of the *Anura* consists of three epithelial parts and a neural part. The lobes of epithelial origin are the anterior lobe proper, the pars intermedia and the pars tuberalis. The pars intermedia is always conformed to the extent of the neural lobe.

In reptiles, birds and mammals the pars intermedia is a thin epithelial layer applied to the neural lobe and is derived from the supradorsal wall of Rathkes' pocket. Later in life the pars intermedia invades the tissue of the neural lobe to a considerable extent. In the frog and toad, also, the pars intermedia conforms to the neural lobe in shape and the two protrude in a bulging fashion on either side of the anterior lobe.

MATERIAL AND TECHNIQUE.

The material used in this experiment was collected near Lawrence, Kan., during the spring and summer of 1918. The writer performed almost all of the operations. The thyroid was removed

when the larva was about five millimeters long. A few thyroidless *Bufo americanus* and a few control *Bufo americanus* of an advanced age were secured from Prof. B. M. Allen. He also placed at my disposal several prepared slides of the hypophysis. The thyroidless specimens used varied from five to twenty millimeters in body length. The control specimens varied from five to almost thirty millimeters in body length. The largest control had completely metamorphosed.

The specimens used for measuring the hypophysis were chosen in pairs—a control and a thyroidless. The total length and body length were used as the deciding criteria for pairing. The criticism might be made that these measurements vary considerably even in a given number of controls of the same age, but when a large number of specimens are used and the averages taken this will not be a great enough factor to affect results seriously. The metamorphosed and nearly metamorphosed *Bufo* were compared with the largest of the thyroidless specimens.

The following measurements were made on all specimens: total length, body length, and leg lengths. They were measured and fixed at different intervals in order to secure a full series from the youngest to the oldest. Bichromate acetic and Zenker's preserving fluids were used. After running the specimens up to 70 per cent alcohol the brains were carefully dissected out and five measurements were made on the hypophysis. These measurements will best be understood by referring to figure 1. This phase of the work might be called a gross measurement study and the second phase a microscopic study.

For the microscopic study the sections were cut in a saggital plane from five to ten microns thick and stained with Heidenhains iron-alum hæmatoxylin. Hæmalum with an eosin counter stain was also used for a few of the slides.

In the microscopic study the three lobes were easily differentiated and every fifth or tenth section projected by a camera lucida on paper ruled into square millimeters. These squares were counted, divided by the magnification and multiplied by the thickness of the section and the number of sections. By this method the volume of each gland was ascertained. This also acted as a check on the gross measurements and shows in a graphic manner the size differences in the different lobes as well as size differences in comparing the controls with the operated specimens. This method is the well-known "Hammar paper method."

RESULTS.

A. GROSS MEASUREMENT STUDY.

Five measurements were made on the hypophysis of eighty-five thyroidectomized *Bufo americanus* and upon seventy-five control *Bufo americanus*.

The removal of the thyroid gland prevents metamorphosis and causes the tadpole to retain its larval characters in spite of the fact that it grows in size. The effects of the extirpation can be detected quite early in the incomplete development of the hind legs as compared with those of a control of a corresponding age. In *Bufo* this

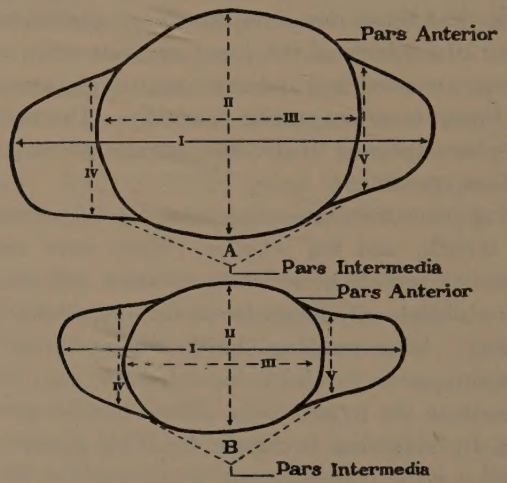

FIG. I. Cameria lucida outline drawing of the entire hypophysis of a thyroidectomized tadpole (A), body length 16.2 mm., and a metamorphosed control (B), body length 28.2 mm. Roman numerals indicate measurements. A Bausch & Lomb 3 mm. objective and ocular 10 were used.

difference is not quite so pronounced as in *Rana*. In the former the body factors which have an effect on metamorphosis exert their influence for a greater length of time in the absence of the thyroid (Allen).

In five- and six-millimeter operated larvæ of *Bufo* the incomplete development of the hind limbs are noted. In many cases they were mere buds. Also the measurements of the hypophysis of the same specimens showed a slight increase over the controls. This increased as size increased.

The accompanying Figure 1 will serve to illustrate the position of the five measurements made on every hypophysis studied and at the

same time will show graphically the immense enlargement that results from the thyroidectomy.

Figure A is the hypophysis of a thyroidectomized *Bufo americanus* and Figure B of a metamorphosed *Bufo americanus*. The length of the body of the operated specimen was only 16.2 millimeters as compared with the metamorphosed control, whose body length was 28.2 millimeters. In spite of the difference in body size, the hypophysis of the thyroidless *Bufo americanus* was considerably larger in all of the measurements.

TABLE I.—The average body and hypophysis measurements of most of the specimens studied. Each group represents the average measurements of at least ten control *Bufo americanus* and ten thyroidectomized *Bufo americanus*.

Pairs of Specimens.	Body measurements in mm.			Hypophysis measurements in mm.				
	Total length.	Body length.	Hind leg length.	Horizontal measurement of anterior lobe. 1	Vertical measurement of anterior lobe. 2	Horizontal measurement of pars intermedia. 3	Vertical measurements of pars intermedia. 4	5
Control	12.1	5.5	.2132	.0792	.0847	.1968	.0382	.0382
Thyroidless	11.0	5.5	.0710	.0874	.0902	.1612	.0464	.0464
Control	16.4	6.8	.7579	.1180	.1204	.2861	.0475	.0475
Thyroidless	13.4	6.8	.2640	.1173	.1246	.2261	.0638	.0656
Control	17.1	7 6	.9713	.1371	.1379	.3045	.0519	.0515
Thyroidless	18.2	7.6	.5774	.1678	.1692	.3087	.0745	.0753
Control	18.9	8.2	5.3020	.1625	.1617	.3564	.0627	.0685
Thyroidless	17.7	8.3	.6835	.1968	.1944	.3481	.0756	.0857
Control	19.9	9.4	5.9329	.1618	.1752	.3578	.0738	.0688
Thyroidless	19.8	9.4	1.5370	.1922	.1916	.3429	.0828	.0879
Control	19.3	10.0	4.568	.1581	.1667	.3365	.0686	.0916
Thyroidless	22.1	10.4	1.885	.1863	.1901	.3280	.1048	.0879
Control	22.3	11.2	6.7258	.1822	.1550	.3660	.0775	.0730
Thyroidless	24.4	11.3	1.3390	.2012	.1960	.3369	.0961	.0984
Control	21.0	12.0	9.2820	.1541	.755	.4182	.0803	.0803
Thyroidless	25.8	12.4	1.5450	.2230	.2676	.3552	.1049	.0918

As suggested before, the increase in size of the hypophysis is noticeable in the youngest specimens and it becomes more pronounced as older and older *Bufo americanus* are studied. These measurements were all made by means of a micrometer eyepiece at the same magnification. The average lengths of the forelegs were omitted because a very few of even the eleven- and twelve-millimeter specimens showed the first appearance of a forelimb.

Several of the operated specimens lived a few months past the normal time for metamorphosis. In comparing the hypophysis of these with the hypophysis of metamorphosed and nearly metamorphosed controls, the size differences were so marked that the comparison could readily be made without the aid of a microscope.

TABLE II.—A few representative pairs out of the dozen or more such pairs of the larger specimens measured.

PAIRS OF SPECIMENS.	Body measurements in mm.				Hypophysis measurements in mm.				
	Total length.	Body length.	Fore leg length.	Hind leg length.	Horizontal measurement of anterior lobe. 1	Vertical measurement of anterior lobe. 2	Horizontal measurement of pars intermedia. 3	Vertical measurements of pars intermedia. 4	5
Control 88.....	14.1	14.1	5.9231	10.684	.1558	.1394	.2870	.0656	.0656
Thyroidless 41...	37.0	14.0	.....	9.328	.3280	.4100	.0984	.1312	.1312
Control 44.....	14.0	14.1	5.684	10.132	.1476	.1394	.2788	.0574	.0574
Thyroidless 2....	30.1	14.2	.....	6.000	.2870	.2788	.4100	.3608	.3608
Control 81.....	22.0	22.0	15.000	30.200	.3526	.4592	.9348	.1722	.1722
Thyroidless 107..	41.8	16.2	.....	6.400	.5740	.4920	.6704	.1640	.1640
Control 80.....	19.0	19.6	12.500	25.500	.3198	.4428	.9020	.1640	.1640
Thyroidless 101..	41.8	19.0	.....	4.510	.7954	.8528	.1024	.3362	.3562
Control 91.....	26.3	26.3	18.200	33.900	.1344	.3790	.6642	.0984	.0984
Thyroidless 98...	43.8	20.0	.....	3.280	.8282	.7872	1.2628	.4592	.4920
Control 86.....	23.0	meta	13.500	25.800	.3280	.3690	.7134	.1640	.1804
Thyroidless 96...	42.0	16.4	.....	5.000	.5248	.6150	.9666	.2050	.2050

In these gross measurements the neural lobe could not be measured except as associated with the pars intermedia. The only successful way to measure the neural lobe is by means of microscopic sections. As previously explained the neural and intermediate lobes are very closely associated. In *Bufo americanus* especially, the neural lobe protrudes slightly beyond the intermediate, but it can only be detected upon exceedingly careful differentiation. This lobe is also comparatively late in development. It does not make its appearance in *Rana* until the larva is about eighteen millimeters in body length (Atwell). It probably develops somewhat earlier in *Bufo*.

The two measurements on the anterior lobe are fairly simple to make, but the measurements on the intermediate lobe offer a more difficult problem. In the averages of the third measurement, namely, across the length of this lobe, a discrepancy often appears. This measurement in the control is sometimes as long as the same measurement in the thyroidectomized specimen. This is usually true only in younger specimens and is probably due to the fact that the pars intermedia bulges out beyond the anterior lobe rather irregularly. This seems to be especially true in the operated specimens and probably at first the growth increase is more in depth than length.

B. MICROSCOPIC STUDY.

Now that it has been shown by gross measurement that the pars anterior and pars intermedia enlarge following the removal of the

thyroid gland it becomes of interest to test the same point by a study of serial sections of normal and thyroidectomized specimens. As suggested, previously, successful gross measurements of the neural lobe cannot be made and therefore especial attention was given to the study of this lobe in the microscopic sections.

The serial sections of twenty-one hypophysis were studied. Of these nine were controls and twelve operated *Bufo americanus* and *Rana pipiens*. For the sake of convenient comparisons, these specimens were divided into three groups, large, medium and small.

The "Hammar paper method" was used for determining the volume of the different lobes.

TABLE III.—Body measurements, volumes of the three lobes of the hypophysis and number of sections in each series of seven control and six thyroidectomized *Bufo americanus*. These were chosen from twenty-one hypophyses studied in serial sections.

	Body measurements in mm.	Volume of anterior lobe.	Volume of intermediate lobe.	Volume of neural lobe.	Number of sections in series.
LARGE.					
Control *Bufo* 17.15	Body length..... 11.9 Total length..... 11.9	.004250	.001420	.002285	36
Control *Bufo* 16.17	Body length..... 11.7 Total length..... 11.7	.001510	.000988	.0008639	50
Control *Bufo* 17.16	Body length..... 11.0 Total length..... 11.0	.003400	.002092	.001164	37
Thyroidless *Bufo* 19.76	Body length..... 21.6 Total length..... 43.4 Hind leg length..... 5.3	.198585	.086750	.021180	127
MEDIUM.					
Control *Bufo* 17.19	Body length..... 10.1 Total length..... 20.8	.002412	.000954	.001085	33
Control *Bufo* 17.18	Body length..... 11.6 Total length..... 23.5	.002800	.001600	.001185	30
Tyroidless *Bufo* 19.86	Body length..... 18.2 Total length..... 23.5	.018000	.014607	.003772	63
Thyroidless *Bufo* 19.71	Body length..... 19.0 Total length..... 43.9	.043510	.019025	.002890	71
Thyroidless *Bufo* 19.72	Body length..... 19.1 Total length..... 43.0	.030451	.015839	.006693	86
SMALL.					
Control *Bufo* XVIII	Body length..... 9.2 Total length..... 23.0	.001520	.000885	.001015	45
Control *Bufo* II	Body length..... 27.0 Total length..... 11.0	.002085	.001255	.001285	45
Thyroidless *Bufo* XXI	Body length..... 9.5 Total length..... 22.0	.008135	.007985	.001055	65
Thyroidless *Bufo* VI	Body length..... 11.2 Total length..... 27.9	.016340	.006185	.000756	61

TABLE IV.—Body measurements, volumes of the three lobes of the hypophysis and number of sections in each series of two control and two thyroidectomized *Rana pipiens*.

	Body measurements in mm.	Volume of anterior lobe.	Volume of intermediate lobe.	Volume of neural lobe.	Number of sections in series.
Control *Rana* XI	Body length..... 14.0 Total length..... 17.2	.001599	.001326	.001525	45
Control *Rana* B	Body length..... 21.8 Total length..... 24.3	.003950	.003775	.003795	57
Thyroidless *Rana* IX	Body length..... 25.0 Total length..... 41.2	.0115695	.008269	.0011329	106
Thyroidless *Rana* No. 3	Body length..... 20.1 Total length..... 40.3	.016160	.018656	.003275	77

In a comparison of control XVIII and thyroidless XXXI the difference in volume of the anterior lobe and pars intermedia is already quite noticeable. These were the youngest specimens studied in microscopic sections. In the older specimens the enlargement of the two lobes was simply enormous. Plates XXVII and XXVIII are the camera lucida drawings of sections of the hypophysis of a metamorphosed *Bufo americanus* and a thyroidectomized *Bufo americanus*. The control is specimen number 17.15 in Table III and the thyroidectomized specimen is number 19.71 in the same table. The body length of the control was 11.9 millimeters and the same measurement for the operated specimen was 19.9 millimeters. These were all drawn at the same magnification and bring out very clearly the size comparisons of the different lobes and especially the enlargement of the lobes in the thyroidectomized *Bufo americanus*.

Every fifth section in the series was drawn. The hypophysis of the control consisted of thirty-six sections and of the operated there were seventy-three sections, each cut ten micra thick.

In all sections of the hypophysis of the controls the three lobes were decidedly easy to differentiate. The anterior lobe was the largest in every case. In the majority of sections the pars intermedia was as large as the neural lobe or slightly larger. There is probably very little actual-size difference in the two.

In all the serial sections of the operated specimens there were several more sections than in controls of a corresponding age. This shows an actual-size increase. In all but one of the thyroidectomized specimens (Rana No. 3) the anterior lobe has a greater volume than the intermediate lobe, but the intermediate lobe is especially large when compared with the same lobe of a control specimen.

The neural lobe is very difficult to differentiate in the operated tadpoles. In many sections it was almost impossible to distinguish

a trace of neural material. In many sections, if there was such material it was loose and scattered along the margin of the infundibulum and not collected into a mass which could be differentiated as a lobe.

The pars intermedia apparently occupies the position of the combined intermediate and neural lobe of the control. Undoubtedly the neural lobe, because of its normally late development and because of the interference of this normal development by thyroidectomy, does not develop to any appreciable extent. Conditions comparable to this are noticed in the study of other structures in thyroidless tadpoles.

CONCLUSIONS.

1. The extirpation of the thyroid gland causes the hypertrophy of the anterior lobe proper and the pars intermedia of the hypophysis in *Bufo americanus* and *Rana pipiens*.

2. Thyroidectomy causes a continuation of larval characteristics in the amphibia and hence the neural lobe is hindered in its growth because of its normally late development. Probably in the oldest thyroidectomized specimens material from the enlarged intermediate lobe has migrated into the small amount of neural tissue present and for that reason the two appear almost inseparable in many sections.

3. The neural lobe is very distinct in the controls. The pars intermedia and neural lobe are of about the same size. The intermediate lobe may be somewhat larger. The anterior lobe is considerably larger than either of these two.

4. The anterior lobe of the thyroidectomized *Bufo americanus* which has lived past the normal time for metamorphosis is at least twice as large as the pars intermedia, and the pars intermedia several times larger than the neural lobe. The anterior lobe and pars intermedia of the thyroidectomized *Bufo americanus* are several times larger than the corresponding lobes of a normal *Bufo americanus* of the same size.

I wish here to express my appreciation to Prof. B. M. Allen, formerly of the University of Kansas, now at the Southern Branch of the University of California, at whose suggestion this problem was undertaken. His help and encouragement made this paper possible. I also wish to thank Dr. H. H. Lane for kindly criticism while writing the final draft of this paper.

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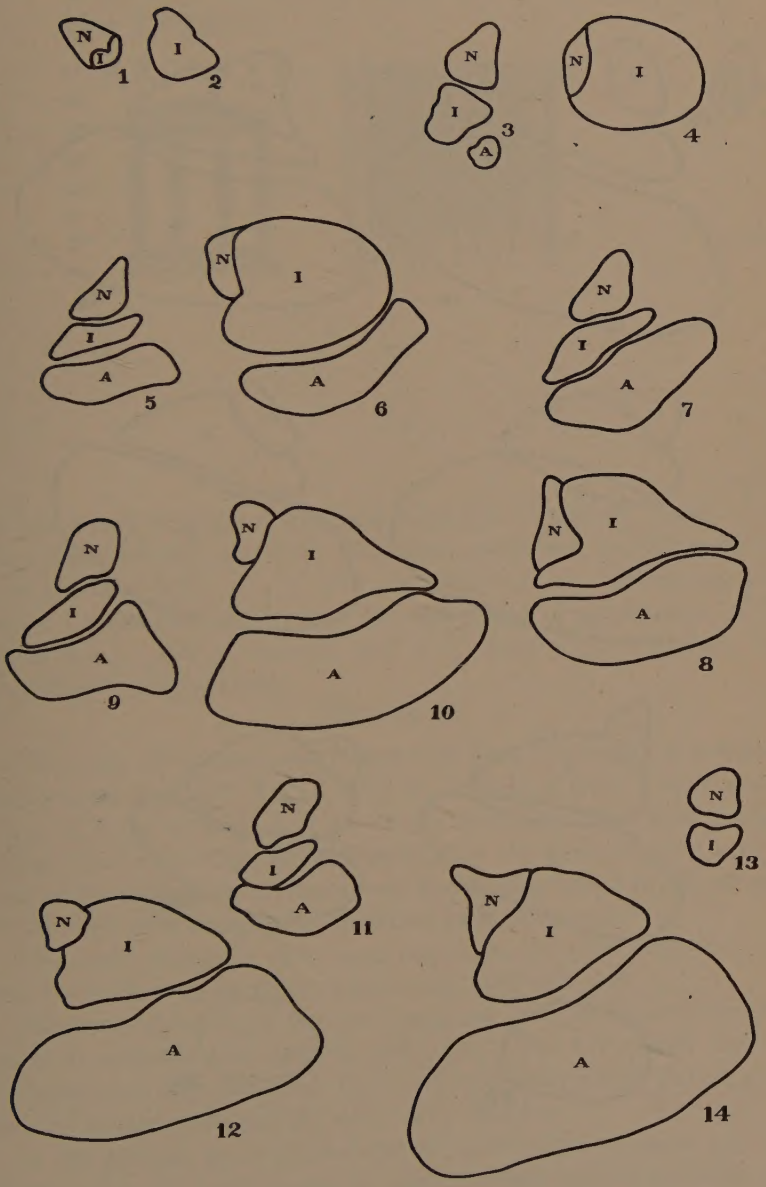
PLATES XXVII AND XXVIII.

Drawings of every fifth section of the hypophysis of a metamorphozed *Bufo americanus* (17.15 in Table III) and a thyroidectomized *Bufo americanus* (19.71 in Table III). The odd numbers represent sections of the hypophysis of the control, while the even numbers represent sections of the thyroidectomized specimen. This arrangement was thought best in order to show at a glance the comparison between the two. Section XV is the last one for the control, but due to the increase in size of the hypophysis of the thyroidectomized *Bufo americanus* there are several more of the latter. As suggested in the text, the neural lobe is quite difficult to differentiate in a thyroidectomized specimen, and so in the drawings it is always shown in close relationship with the intermediate lobe. In the control the line of demarcation is very distinct.

All figures were drawn with the aid of a camera lucida at table level. A Bausch & Lomb 3 mm. objective and ocular 10 were used.

Abbreviations: A, anterior lobe; N, neural lobe; I, intermediate lobe.

PLATE XXVII.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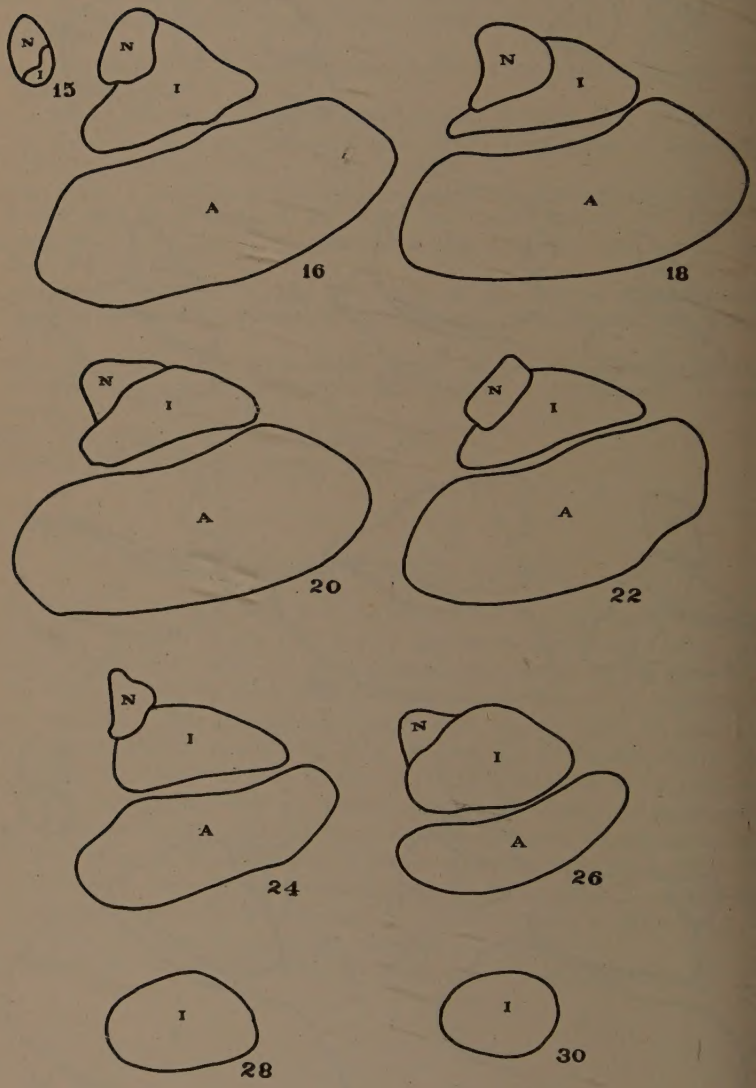
PLATE XXVIII.